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**Development of an Efficient Transformation System for
*Dothistromin pini***

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Abstract

A transformation system has been developed for the plant pathogenic fungus *Dothistroma pini* using a positive selection system based on the *Escherichia coli* hygromycin B phosphotransferase gene (*hph*). After optimising the conditions under which protoplasts were isolated a transformation protocol was determined. The system developed gave large, stable transformants at frequencies between 1 and 48 transformants per μg of DNA. A second type of colony also grew on the selective plates. These grew in higher numbers but less vigorously, and did not grow when subcultured onto plates containing hygromycin B. These are believed to be abortive transformants. Southern analysis indicated that transformation takes place via the integration of the plasmid DNA into the fungal chromosomal DNA. The DNA integrated at a single site in 88% of the transformants, with all of the sites containing only a single copy of pAN7-1. Propagation of two of the transformants through single spore analysis indicated that they were homokaryons, though molecular results of another transformant indicated that it was a heterokaryon. Placing the transformants on increasing concentrations of hygromycin B indicated that the copy number of the integrated plasmid was not related to hygromycin resistance. In order to try and enhance the transformation rate of *Dothistroma pini*, by using a homologous promoter, the β -tubulin gene was isolated from a genomic library using the β -tubulin gene from *Neurospora crassa* as a probe. A restriction map was made and the gene was sequenced and shown to closely resemble β -tubulin genes from other fungi.

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